REMARKS

Applicants submit herewith the Exhibits, which were not received with Applicants' response filed April 15, 2005. The relevant portions of Applicants' reply relating to the Exhibits are excerpted below for the Examiner's convenience:

> The anti-phosphotyrosine antibody disclosed by Buday is the 4G10 antibody sold by Upstate (A certificate of analysis of the 4G10 antibody is attached hereto as Exhibit A.) Hirth does not disclose which anti-phosphotyrosine antibody was used but only that an anti-phosphotyrosine serum was used (Col. 25, lines 26-27).
>
> It is well known that anti-phosphotyrosine antibodies do not bind to every phosphorylated tyrosine of every protein. That is, it is well known that anti-phosphotyrosine antibodies have specificity and selectivity. Thus, for instance, attached as Exhibits A and B are copies of certificates of analysis of two different anti-phosphotyrosine antibodies sold by Upstate, the cited 4G10 antibody and a rabbit polyclonal anti-phosphotyrosine antibody mixture. These certificates of analysis show that under the same conditions, different proteins were recognized by the different antibodies. In turn, these certificates of analysis show that not every tyrosine phosphorylated antibody will recognize the same proteins, i.e., the same phosphorylated tyrosines.

Yet further, attached as Exhibit C is a product insert for Zymed Laboratories PY-Plus Mouse anti-Phosphotyrosine (Cocktail) product. Under the heading "Reactivity" the product insert reads, "[i]t is well established that no one monoclonal antibody can react with all tyrosine phosphorylated proteins."

In view of such evidence, it can not be assumed or even expected that antibodies reported by Buday and Hirth would "specifically bind to a LAT polypeptide comprising an amino acid sequence according to SEQ ID NO: 4."

It is believed the application is in conditions for immediate allowance, which action is earnestly solicited.

A separate Petition for Extension of Time is also submitted herewith for a one (1) month extension of time to May 13, 2005. Applicant also conditionally petitions for a further extension time to provide for the possibility that such a petition is required. Please charge Deposit Account No. **04-1105** for the required fee.

Respectfully submitted,

Date: May 5, 2005

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EXHIBIT A

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Technical Support: T: 800 548-7853 • F: 518 523-4513
email: techserv@upstate.com
Sales Department: T: 800 233-3991 • F: 781 890-7738
Licensing Dept.: 800 310-4659
www.upstate.com

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Certificate of Analysis

Anti-Phosphotyrosine, clone 4G10

(mouse monoclonal $IgG_{2bκ}$)
Catalog # 05-321
Lot # 28818

Immunogen: Phosphotyramine-KLH.

Antibody Class: $IgG_{2bκ}$ mouse monoclonal antibody produced *in vitro* by mouse-mouse hybridoma 4G10 (FOX-NY [NS-1 derivative] myeloma x spleen cells). Purified by Protein G-Sepharose chromatography.

Formulation: **100µg** of protein G purified mouse monoclonal $IgG_{2bκ}$ in **100µl** of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide. Liquid at 4°C.

Storage and Stability: Stable for 2 years at 4°C from date of shipment. **NOTE: DO NOT FREEZE.** For maximum recovery of the product, centrifuge the original vial prior to removing the cap. If the product has accidentally been frozen and thawed, spin it at 13,000 x g for 10 minutes at 4°C. Save the supernatant for application.

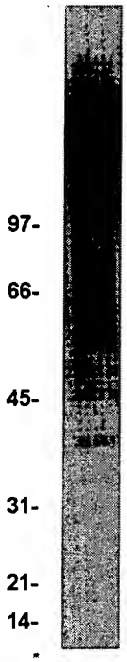
FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected tyrosine-phosphorylated proteins in a modified RIPA lysate from EGF-treated human A431 carcinoma cells.[1,2,3]

Included Positive Antigen Control: Catalog # 12-302, EGF-stimulated A431 cell lysate is provided as a free positive antigen control for western immunoblotting. Aliquot as desired, refreeze immediately, and store at -20°C. The lysate is stable for 6 months at -20°C. Before use, **add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for immunoblot analysis.

Immunoprecipitation: 2-4µg of this lot can immunoprecipitate quantitatively the phosphotyrosine-containing proteins in the lysate of a confluent culture (10cm dish) of cells expressing an activated tyrosine kinase. To preserve phosphotyrosine, add 0.2mM sodium orthovanadate to the lysis buffer.



Immunoblot Analysis:
Representative blot from a previous lot. EGF-stimulated A431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phosphotyrosine (1µg/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

References:

1. Cohen, B., *et al.*, Proc. Natl. Acad. Sci. USA. **87**: 4458-4462, 1990.
2. Druker, B.J., *et al.*, New Eng. J. Med. **321:** 1383-1391, 1989.
3. Kanakura, Y., *et al.*, J. Biol. Chem. **266:** 490-495, 1991.

Immunoprecipitation Protocol

1. Add **2-4μg of anti-Phosphotyrosine, clone 4G10** and 60μl (30μl packed beads) of washed Protein G agarose bead slurry (Catalog # 16-266) to 500μl of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
4. Dilute the cell lysate to roughly 1μg/μl total cell protein with PBS.
5. Add 500μg-1mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 60μl 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1μg/ml aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 45-90 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2μg/ml of anti-Phosphotyrosine, clone 4G10,** diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated, Catalog # 12-349, 1:2000 dilution, was used) in TBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence with a 30 second exposure was used).

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Certificate of Analysis

Anti-Phosphotyrosine

(rabbit immunoaffinity purified IgG)
Catalog # 06-427
Lot # 28136

Immunogens: In order to produce broad spectrum polyclonal phosphotyrosine antibodies, rabbits were immunized with three phosphorylated immunogens: (1) phosphotyrosine covalently linked to KLH; (2) the c-Src carboxyl terminal regulatory phosphopeptide (T-S-T-E-P-Q-pY-Q-P-G-E-N-L; Catalog # 12-218) covalently linked to KLH, and; (3) a phosphopeptide associated with high tyrosine kinase activity in human lymphocytes (R-R-L-I-E-D-A-E-pY-A-A-R-G; Catalog # 12-217) covalently linked to KLH. Both of the phosphopeptide haptens serve as strong substrates for tyrosine phosphatases and are part of the two colorimetric tyrosine phosphatase kits provided by Upstate, Inc. (Catalog # 17-125, 17-126).

Species Cross-reactivity: Human, mouse and rat. Other species cross-reactivity is unknown.

Specificity and Purification: The immunoreactivity of the antibody is totally inhibited by the use of 100mM phenyl phosphate, a phosphotyrosine analog. The phosphotyrosine antibody is purified by immunoaffinity chromatography using either a dual phospho-peptide gel or a BSA-phosphotyrosine gel. All of the phosphotyrosine immunoreactivity present in the antisera is immunoadsorbed whether the antibody is purified by either gel indicating that the antibody is not sequence-specific but specific for phosphotyrosine residues.

Formulation: **200µg** of immunoaffinity purified rabbit IgG in **900µl** of 0.2M Tris-glycine, pH 7.2, 0.15M NaCl, 5mg/ml of BSA containing 0.05% sodium azide. Frozen solution.

Storage and Stability: Stable for 1 year at -20°C from date of shipment. Aliquot to avoid repeated freezing and thawing. For maximum recovery of the product, centrifuge the original vial after thawing and prior to removing the cap.

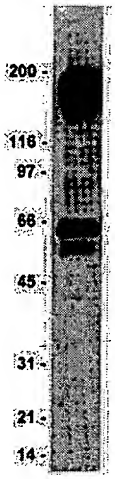
FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 0.5-2µg/ml of this lot detected proteins containing phosphotyrosine residues in RIPA lysates from EGF-stimulated human A431 carcinoma cells.

Included Positive Antigen Control: Catalog # 12-302, EGF-stimulated A431 cell lysate. **Add 2.5µl of 2-mercaptoethanol/100µl of lysate and boil for 5 minutes to reduce the preparation.** Load 20µg of reduced lysate per lane for minigels.

Immunoprecipitation: 4µg of a previous lot immunoprecipitated proteins containing phosphotyrosine residues from a human A431 RIPA lysate.



Immunoblot Analysis
Representative blot from a previous lot. EGF-stimulatedA431 cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-Phosphotyrosine (0.5µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system.

References:

1. The cSrc carboxyl terminal regulatory phosphopeptide (T-S-T-E-P-Q-pY-Q-P-G-E-N-L) which binds to the internal SH2 domain of c-Src.
   Song, Z., *et al.*, Cell **72:**767, 1993.
   Luttrel, D.K., *et al.*, Proc. Natl. Acad. Sci. USA **91:** 83, 1994.
2. The phosphopeptide associated with high tyrosine kinase activity in human lymphocytes (R-R-L-I-E-D-A-E-pY-A A-R-G).
   Trevillyan, J.M., *et al.*, Biochim. Biophys. Acta **845**: 1, 1985.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml aprotinin, leupeptin, pepstatin; 1mM Na_3VO_4; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200) and 0.05% Tween-20 (PBST-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2µg/ml of anti-Phosphotyrosine**, diluted in freshly prepared PBST-MLK, overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a **goat anti-rabbit** HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution, was used) in PBST-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Immunoprecipitation Protocol

1. Add **4µg of anti-Phosphotyrosine** and 60µl (30µl packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500µl of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 1 hour.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
4. Dilute the cell lysate to roughly 1µg/µl total cell protein with PBS.
5. Add 500µg-1mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 60µl 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

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Qty: 100 µg/200 µl

PY-Plus™ Mouse anti-Phosphotyrosine (Cocktail)

Catalog No. 13-6600

Lot No.

PY-Plus™ Mouse anti-Phosphotyrosine (Cocktail)

FORM
PY-Plus™ is supplied as a 200 µl aliquot at a concentration of 0.5 mg/ml in PBS, pH 7.4, containing 0.1% sodium azide. PY-Plus™ contains equal amounts of anti-phosphotyrosine monoclonal antibodies PY-7E1 and PY20. Each antibody is highly purified from mouse ascites by phosphotyrosine-specific affinity chromatography prior to blending.

CLONES: PY-7E1, PY20

ISOTYPES: IgG_1-κ, IgG_{2b}

SPECIFICITY
PY-Plus™ reacts specifically with tyrosine-phosphorylated proteins. It shows no cross-reactivity to phosphoserine or phosphothreonine. On Western blots, specific inhibition of each individual antibody and the cocktail reactivity can be achieved by pre-incubation of the antibody with 20 mM phosphotyrosine, whereas pre-incubation with 20mM phosphoserine or 20 mM phosphothreonine is ineffective.

REACTIVITY
It is well established that no one monoclonal antibody can react with all tyrosine phosphorylated proteins (on Western blots of A431 cell lysates, PY-7E1 and 4G10 display almost identical band patterns while PY-20 is significantly different. PY-Plus™ contains 2 different monoclonal antibodies that are designed to strongly react with the broadest range of tyrosine-phosphorylated proteins possible. It will react with tyrosine-phosphorylated proteins in both the native and denatured states. Positive reactivity is independent of the species source and has been observed in the following samples: mouse brain, NIH 3T3 cells (+/- TPA), K562 cells, EGF-stimulated A431 cells, and MCF-7cells.

USAGE
The dilutions below are only starting recommendations. Optimal concentrations of this antibody should be determined by the investigator for each specific application.

Western Blotting[8,9]**:**	1 µg/ml
Immunoprecipitation[10]**:**	5 µg

STORAGE
Store at 2-8°C for up to one month. Store at –20°C for long term storage. Avoid repeated freezing and thawing.

BACKGROUND:[1-7]
Protein tyrosine phosphorylation plays a central role in the transmission of signals from the cell surface to the nucleus.[5,7] Reversible phosphorylation of cellular proteins on specific tyrosine residues is involved in a variety of fundamental cellular processes including survival, proliferation, differentiation, and transformation.[4,5,7] Moreover, the use of antibodies which specifically recognize phosphotyrosine is widely accepted as one of the most powerful tools for detection, characterization and purification of proteins which are either constitutively or inducibly phosphorylated on tyrosine (where even a single phosphorylated tyrosine residue is sufficient for detection).[1,2,3,6]

(cont'd)

REFERENCES

1. Daniel, T.O., et al; *Proc. Natl. Acad. Sci. USA* 82:2684-2687 (1985).
2. Frackelton, A.R., et al; *Mol. Cell. Biol.* 3:1343-1352 (1983).
3. Glenny, J.R., et al; *J. Immunological Meth.* 109:277-285 (1988).
4. Hunter, T., et al; *Methods in Enzymology* 200:3-37 (1991).
5. Hunter, T., et al; *Cell* 50:823-829 (1987).
6. Sengupta, A., et al; *Proc. Natl. Acad. Sci. USA* 85:8062-8066 (1988).
7. Sun, H., et al; *Trends Biochem. Sci.* 19:480-485 (1994).
8. Bourguignon, L.Y., et al; *J. Biol. Chem.* 272(44):27913-27918 (1997).
9. Hua, C.I., et al; *J. Biol. Chem.* 273(43):283332-28340 (1998).
10. Conus, N.M. et al; *J. Biol. Chem.* 273(8):4776-4782 (1998).

RELATED PRODUCTS

Product	*PAD*/Clone*	*Cat. No.*	
PY-*Plus*™ Cocktail-HRP	3 mabs	13-6620	
Rb x Phosphotyrosine	Z-PY1	61-5800	
Rb x Phosphotyrosine-HRP	Z-PY1	61-5820	
Rb x Phosphotyrosine-Sepharose®	Z-PY1	61-5841	
Ms x Phosphotyrosine	PY-7E1	13-5900	
Ms x Phosphotyrosine-HRP	PY-7E1	13-5920	
Ms x Phosphotyrosine	PY20	03-7700	
Ms x Phosphotyrosine (1 mg size)	PY20	03-7799	**Outstanding Value!**
Ms x Phosphotyrosine-HRP	PY20	03-7720	
Ms x Phosphotyrosine-AP	PY20	03-7722	
Ms x Phosphotyrosine-Biotin	PY20	03-7740	
Ms x Phosphotyrosine- Sepharose®	PY20	03-7742	
Ms x Phosphotyrosine	Z027	03-5800	
Phosphotyrosine Sampler Pack	5 antibodies	90-0100	
Phosphotyrosine Ab inhibitor	--	79-0003	
Phospho-Amino Acid Sampler Pack (pSer, pThr, PY-Plus™ Cocktail)	2 poly, 1 mab	90-0200	
Rb x Phosphoserine	Z-PS1	61-8100	
Phosphoserine Ab Inhibitor	--	79-0001	
Rb x Phosphothreonine	Z-PT1	71-8200	
Ms x Phosphothreonine	PT-5H5	13-9200	
Phosphothreonine Ab Inhibitor	--	79-0002	

*Polyclonal Antibody Designation

Conjugate	ZyMAX™ Goat x Rabbit IgG (H+L)	ZyMAX™ Goat x Mouse IgG (H+L)
Purified	81-6100	81-6500
FITC	81-6111	81-6511
TRITC	81-6114	81-6514
Cy™3	81-6115	81-6515
Cy™5	81-6116	81-6516
HRP	81-6120	81-6520
AP	81-6122	81-6522
Biotin	81-6140	81-6540

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